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Zou et al.

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(54) **REAL-TIME FOCUSING IN LINE SCAN IMAGING**

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G02B 21/24	(2006.01)
G02B 21/36	(2006.01)

(52) **U.S. Cl.**

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(58) **Field of Classification Search**
None
See application file for complete search history.

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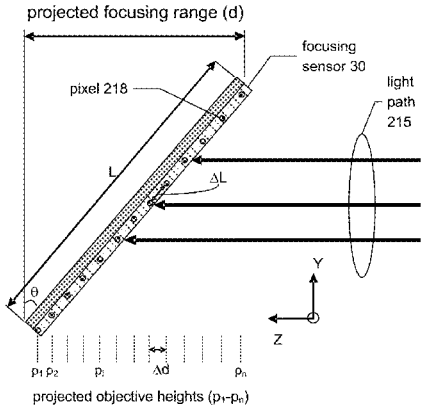
(57) **ABSTRACT**

Systems and methods for capturing a digital image of a slide using an imaging line sensor and a focusing line sensor. In an embodiment, a beam-splitter is optically coupled to an objective lens and configured to receive one or more images of a portion of a sample through the objective lens. The beam-splitter simultaneously provides a first portion of the one or more images to the focusing sensor and a second portion of the one or more images to the imaging sensor. A processor controls the stage and/or objective lens such that each portion of the one or more images is received by the focusing sensor prior to it being received by the imaging sensor. In this manner, a focus of the objective lens can be controlled using data received from the focusing sensor prior
(Continued)



to capturing an image of a portion of the sample using the imaging sensor.

20 Claims, 8 Drawing Sheets

Related U.S. Application Data

(60) Provisional application No. 61/641,788, filed on May 2, 2012.

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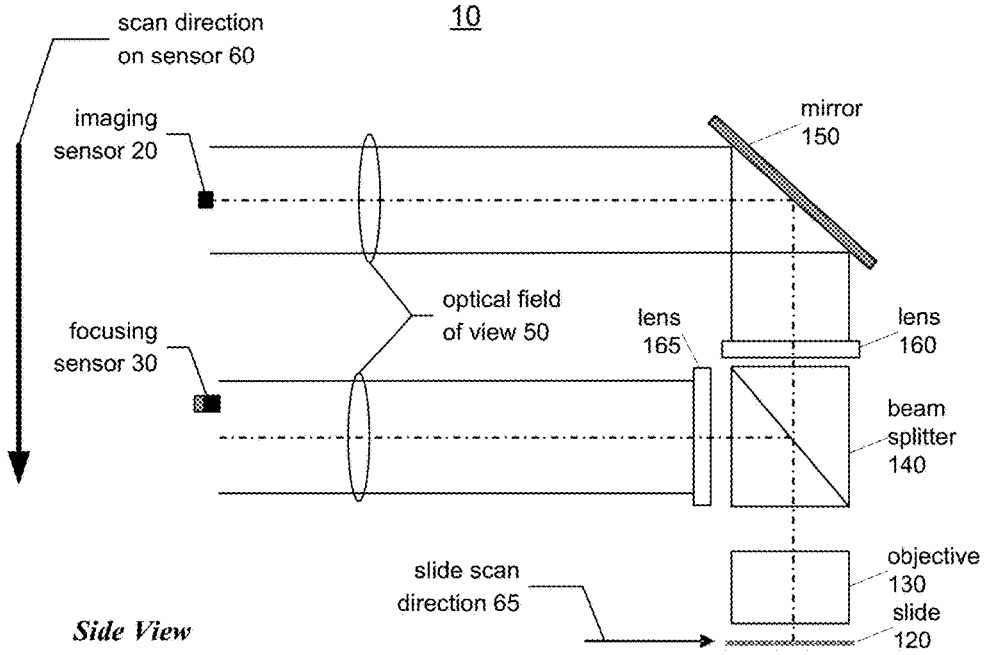

10
scan direction
on sensor 60
imaging
sensor 20
focusing
sensor 30
optical field
of view 50
mirror
150
lens
165
lens
160
beam
splitter
140
objective
130
slide
120
slide scan
direction 65
Side View

FIG. 1

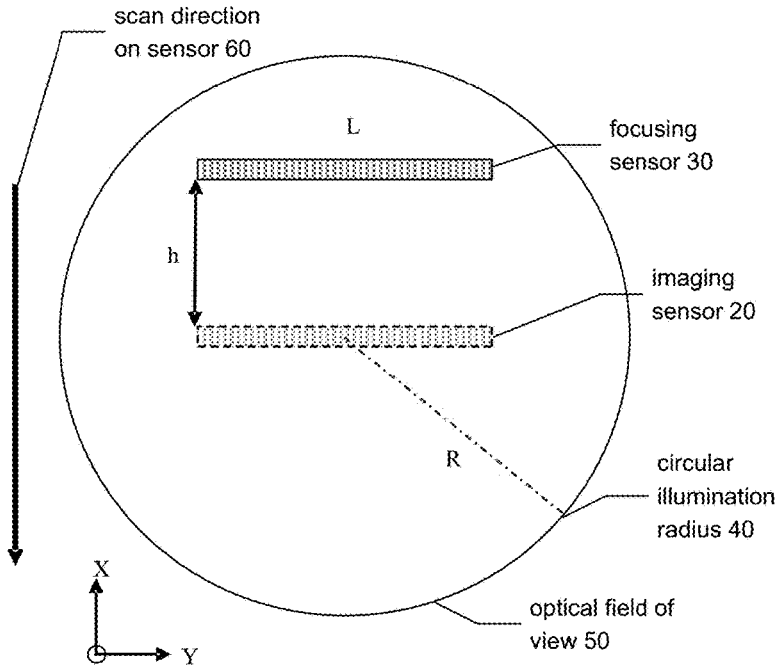
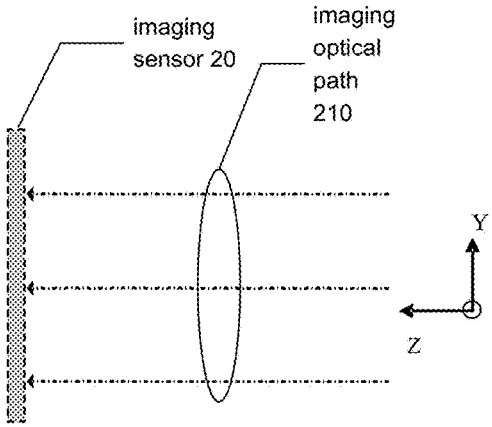
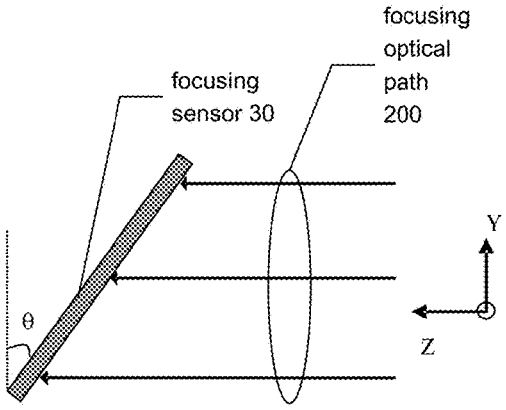

scan direction
on sensor 60
L
focusing
sensor 30
h
imaging
sensor 20
R
circular
illumination
radius 40
optical field of
view 50
X
Y

FIG. 2



Top View

FIG. 3A



Top View

FIG. 3B

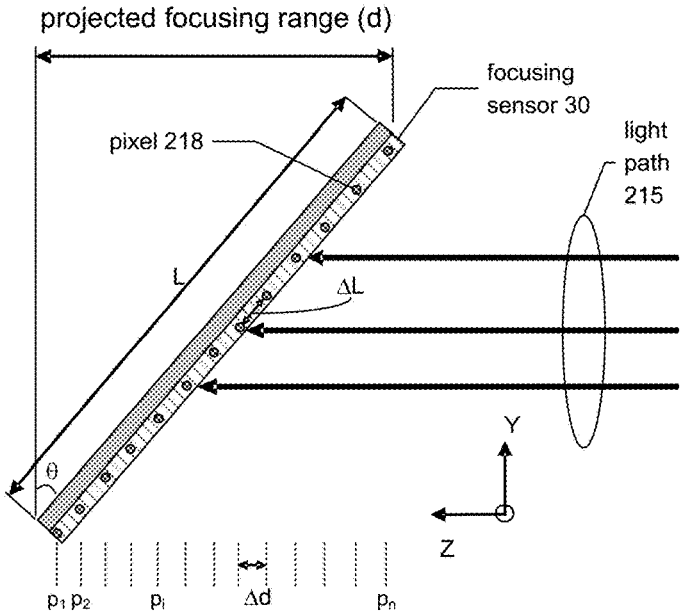


FIG. 4

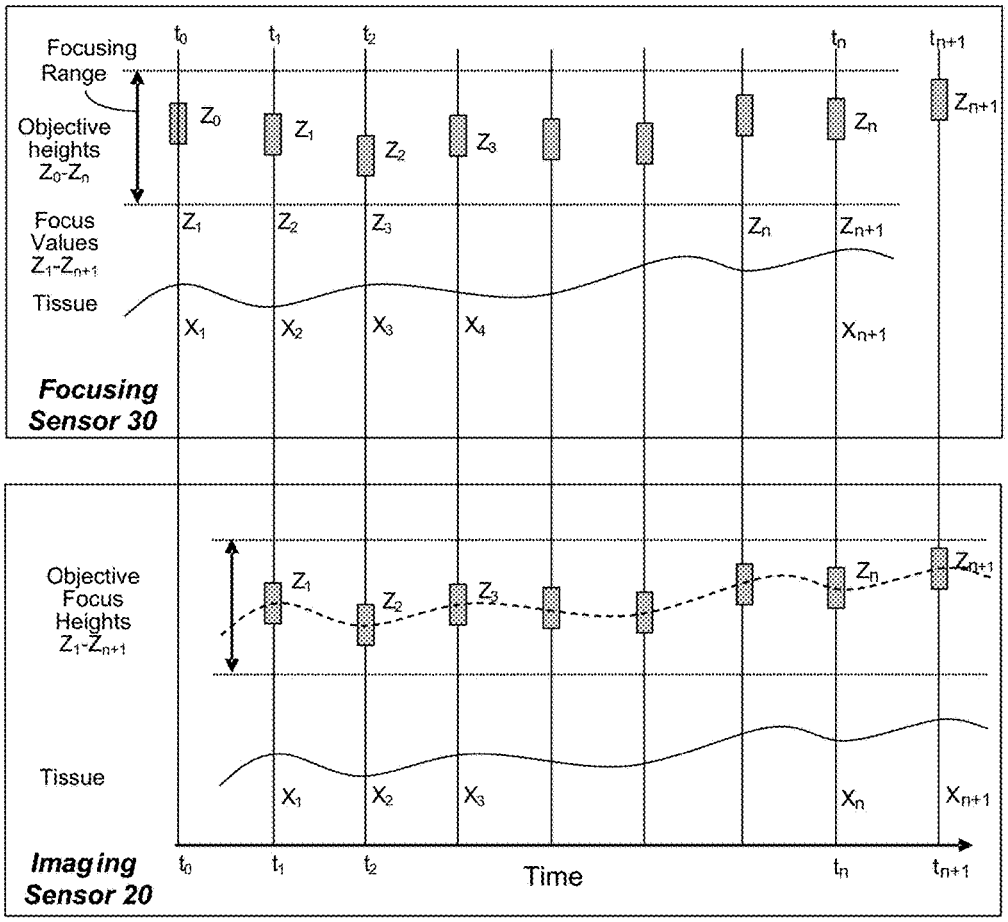

Focusing Range
Objective heights Z_0-Z_n
Focus Values Z_1-Z_{n+1}
Tissue
Focusing Sensor 30
Objective Focus Heights Z_1-Z_{n+1}
Tissue
Imaging Sensor 20
Time

FIG. 5

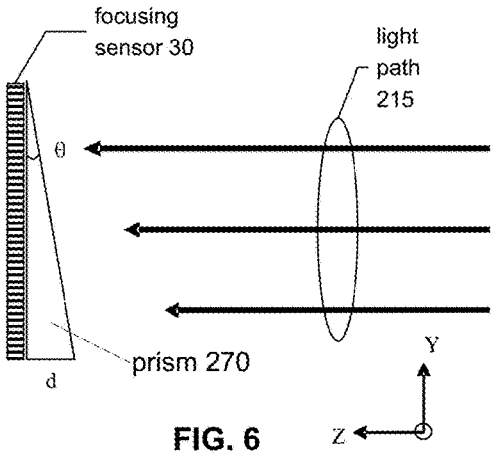

focusing sensor 30
light path 215
θ
prism 270
d
Y
Z

FIG. 6

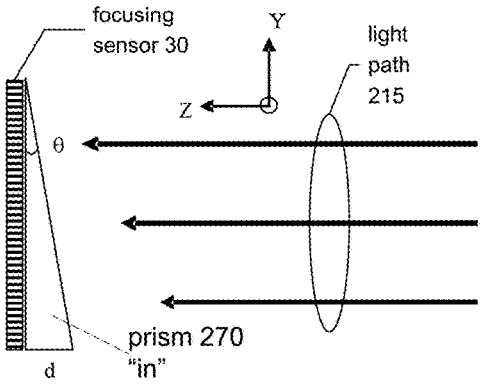


FIG. 7A focusing during stage retrace

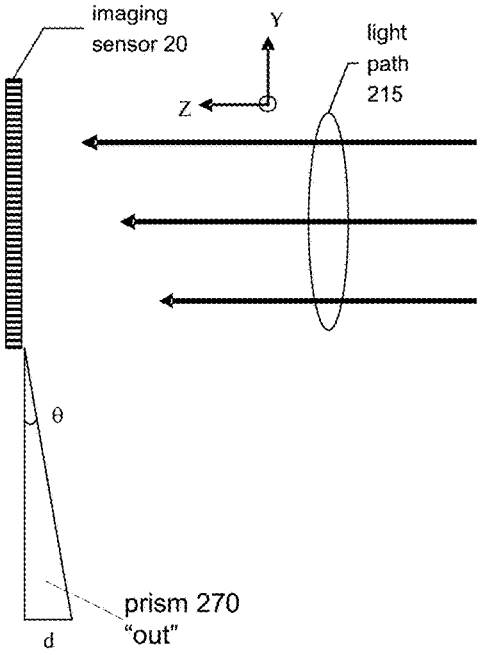


FIG. 7B imaging during scan

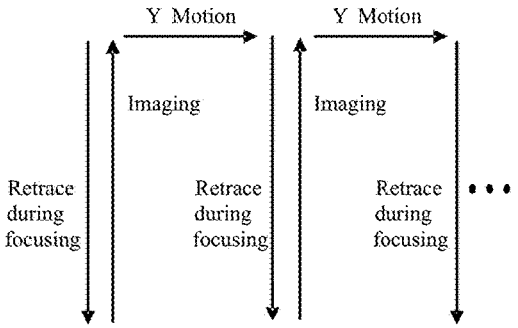


FIG. 9

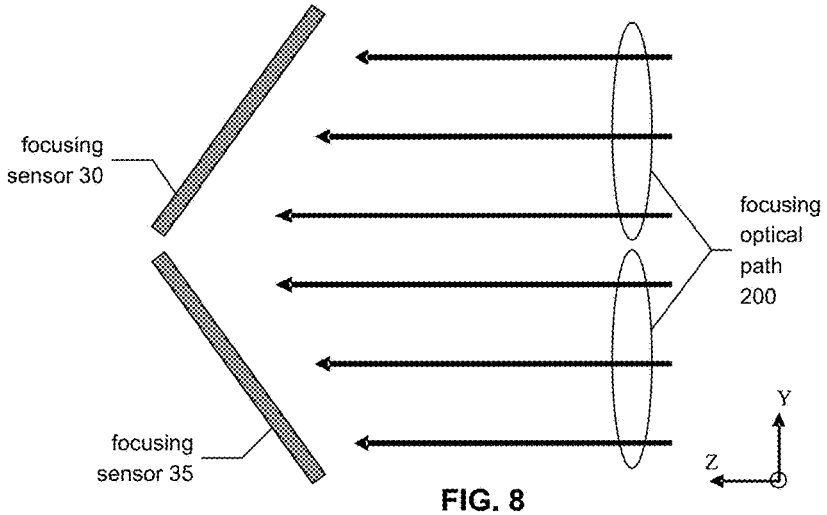


FIG. 8

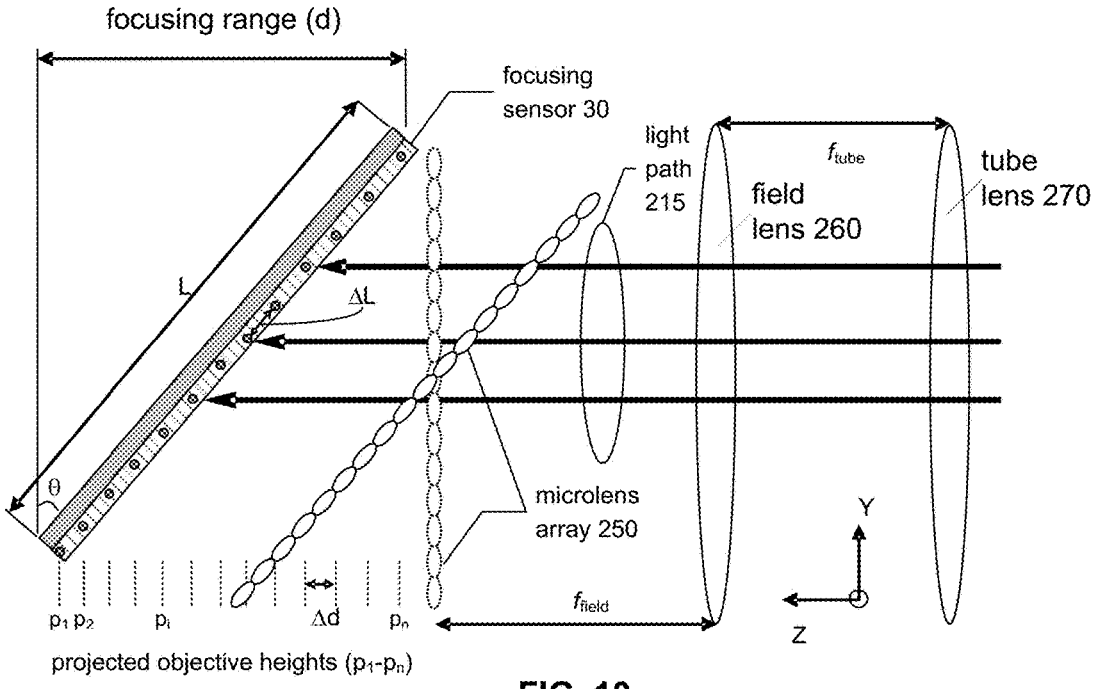

focusing range (d)
focusing sensor 30
light path 215
f_{tube}
field lens 260
tube lens 270
L
ΔL
θ
microlens array 250
Y
Z
p_1 p_2
p_i
Δd
p_n
f_{field}
projected objective heights (p_1-p_n)

FIG. 10

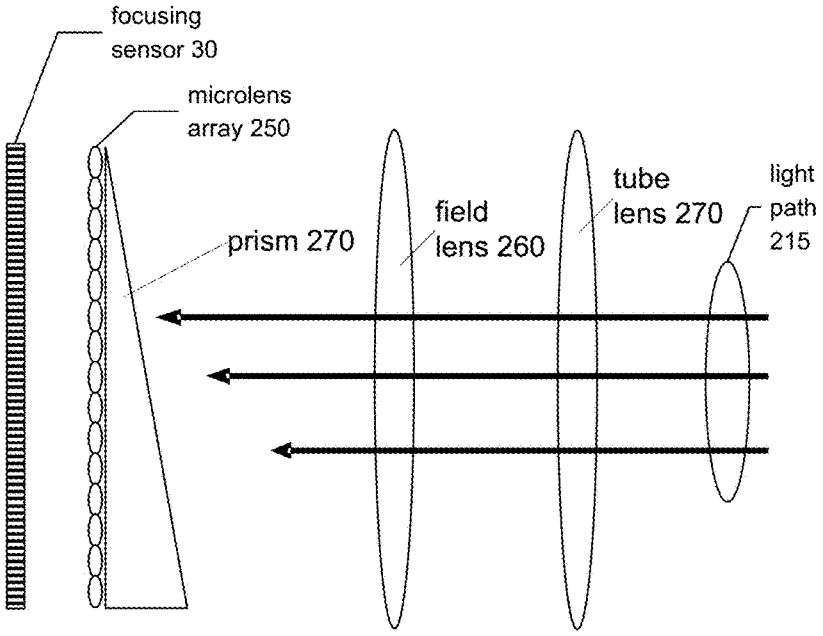

focusing sensor 30
microlens array 250
prism 270
field lens 260
tube lens 270
light path 215

FIG. 11

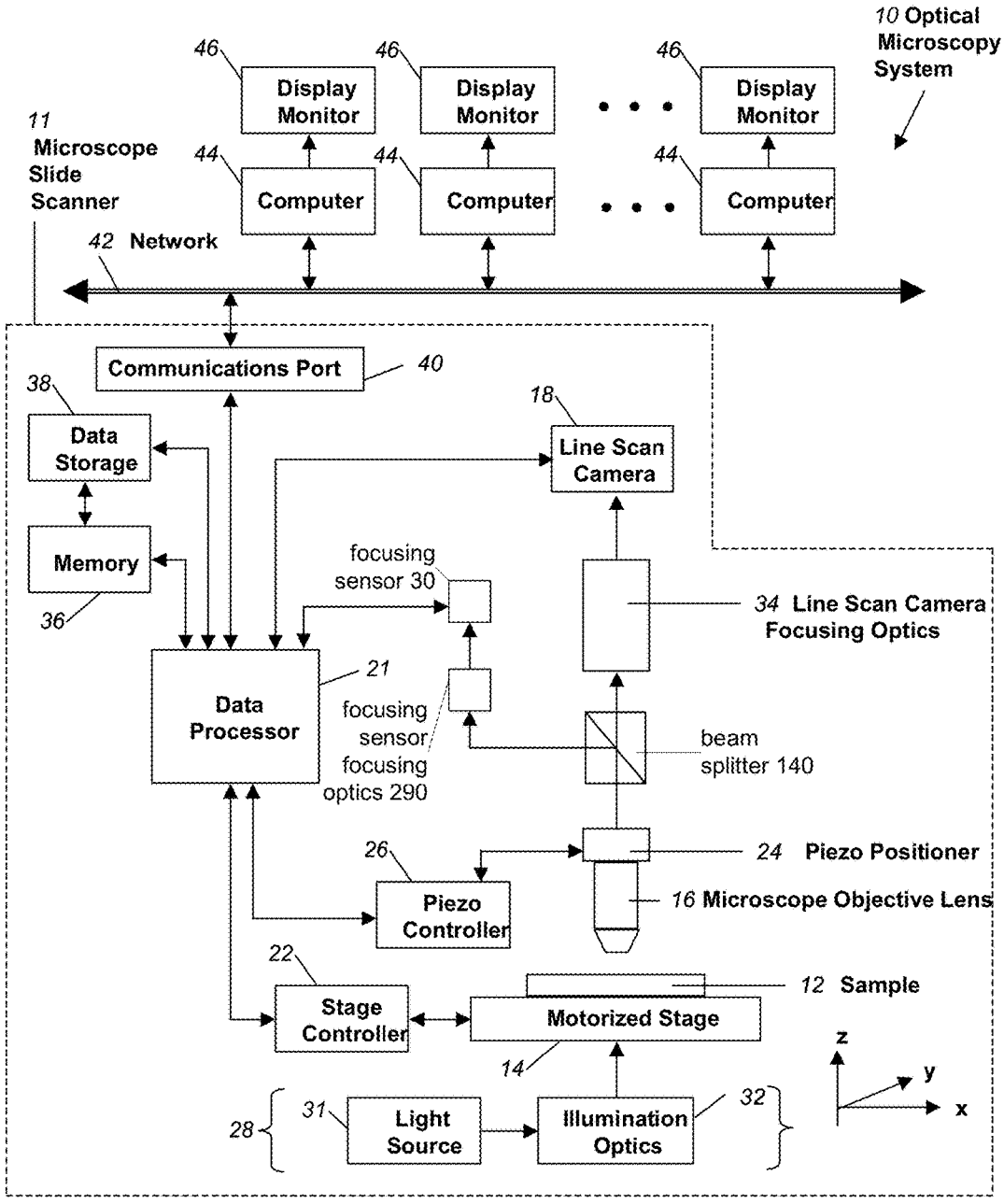

10 Optical Microscopy System
11 Microscope Slide Scanner
46
Display Monitor
44
Computer
42 Network
Communications Port
40
38
Data Storage
Memory
36
Data Processor
21
18
Line Scan Camera
34 Line Scan Camera Focusing Optics
focusing sensor 30
focusing sensor focusing optics 290
beam splitter 140
24 Piezo Positioner
16 Microscope Objective Lens
26
Piezo Controller
22
Stage Controller
12 Sample
Motorized Stage
14
28
31
Light Source
Illumination Optics
32
z
y
x

FIG. 12A

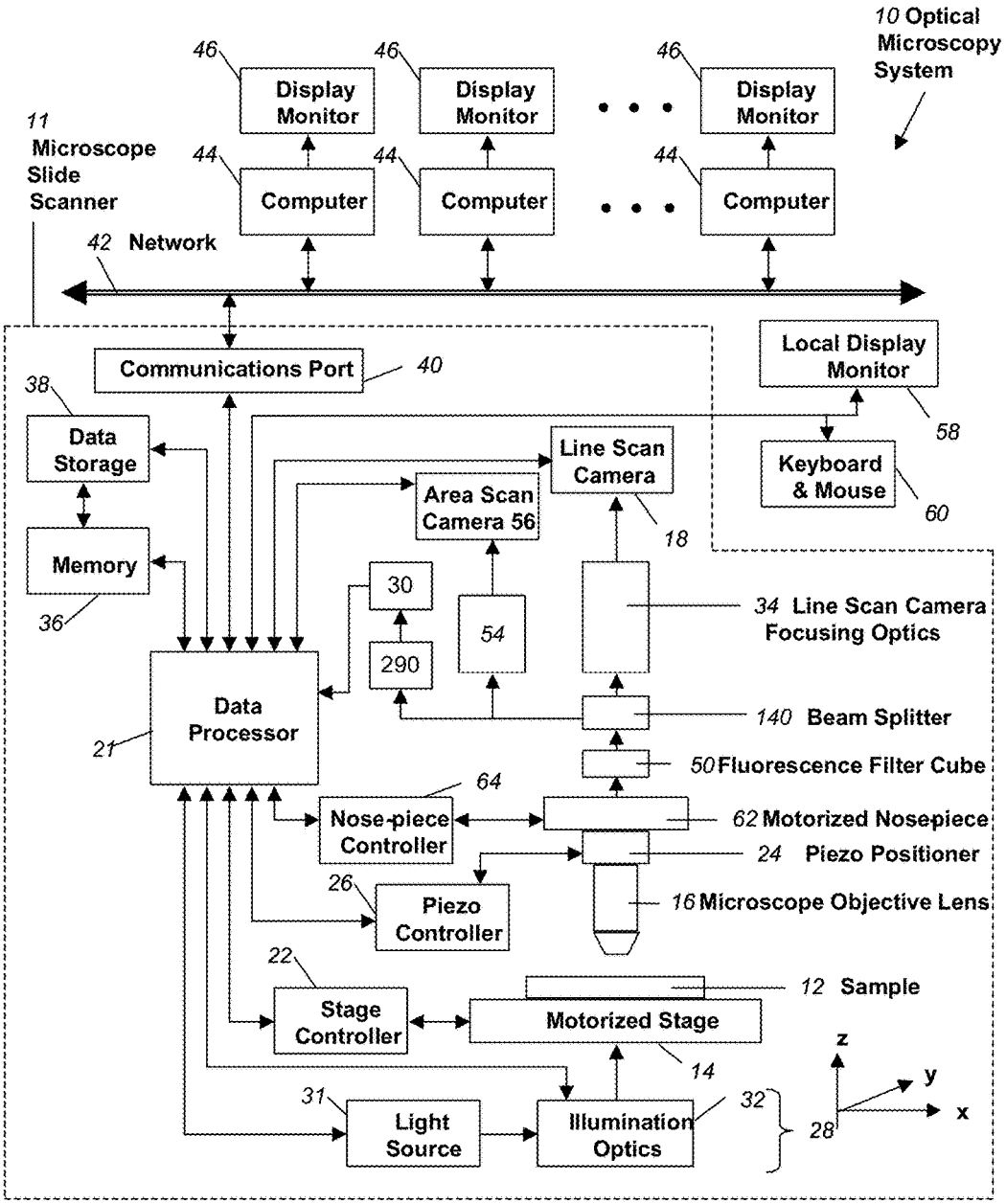

10 Optical Microscopy System
11 Microscope Slide Scanner
46
Display Monitor
44
Computer
42 Network
Communications Port
40
Local Display Monitor
58
38
Data Storage
Keyboard & Mouse
60
Line Scan Camera
18
Area Scan Camera 56
Memory
36
30
290
54
34 Line Scan Camera Focusing Optics
Data Processor
21
140 Beam Splitter
50 Fluorescence Filter Cube
64
Nose-piece Controller
62 Motorized Nosepiece
24 Piezo Positioner
26
Piezo Controller
16 Microscope Objective Lens
22
Stage Controller
12 Sample
Motorized Stage
14
31
Light Source
Illumination Optics
32
28
z
y
x

FIG. 12B

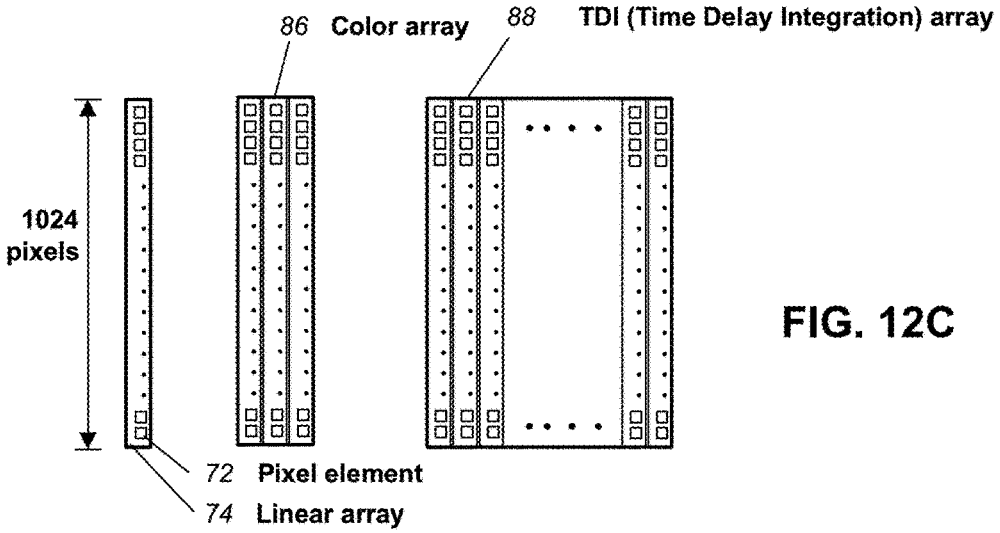


FIG. 12C

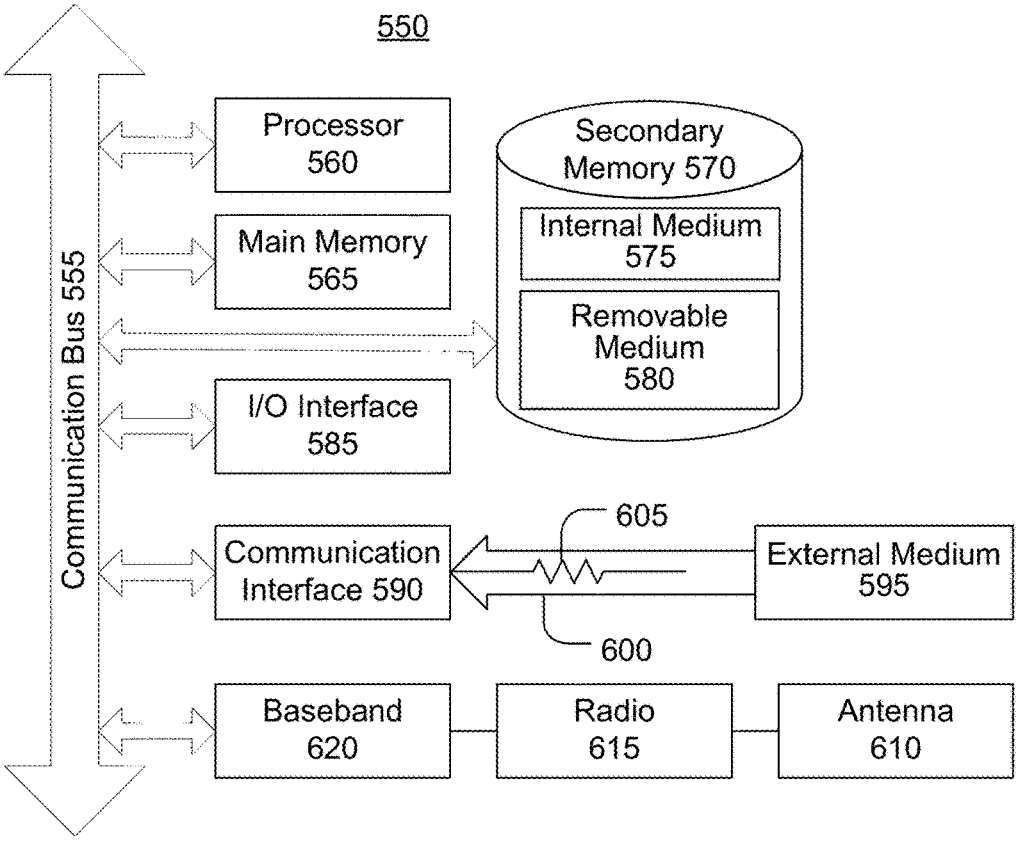


FIG. 13

REAL-TIME FOCUSING IN LINE SCAN IMAGING

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. patent application Ser. No. 14/398,443, filed on Oct. 31, 2014, which is a national stage entry of International Patent App. No. PCT/US2013/031045, filed on Mar. 13, 2013, which claims priority to U.S. Provisional App. No. 61/641,788, filed on May 2, 2012, the disclosures of all of which are hereby incorporated herein by reference as if set forth in their entireties.

BACKGROUND

Field of the Invention

The present invention generally relates to digital pathology and more particularly relates to a multiple independent linear sensor apparatus for performing real-time focusing in line scan imaging.

Related Art

Most auto-focus methods in microscopic imaging systems can be divided into two categories: laser-based interferometer for sensing slide position and image content analysis. The image content analysis methods require multiple image acquisitions at different focus depths and use algorithms to compare the images to determine the best focus. Acquiring the multiple images may create time delays between focusing and imaging. Measuring the reflection of the laser beam off a slide surface can provide only global focus information of the slide or the cover slip position. It lacks focusing accuracy to tissues with large height variations.

Therefore, what is needed is a system and method that overcomes these significant problems found in the conventional systems as described above.

SUMMARY

In an embodiment, the present invention is based on image content analysis (e.g., tissue finding and macro focus), and takes advantage of line imaging and line focusing for accurate real-time auto-focusing, without having to take multiple images for focusing which would introduce a time delay during scanning. In one embodiment, full stripe focusing is performed during a retrace process of line scanning. In an alternative embodiment, focusing is performed during image scanning. Both embodiments eliminate time delays in image scanning, thus speeding up the entire digital image scanning process.

In an embodiment, a system for capturing a digital image of a slide is disclosed. The system comprises an objective lens having a single optical axis; a motorized positioner to control the objective lens; a stage configured to support a sample; at least one imaging sensor; at least one focusing sensor; at least one beam-splitter optically coupled to the objective lens and configured to receive one or more images of at least a portion of the sample through the objective lens, and simultaneously provide a first portion of the one or more images to the at least one focusing sensor and a second portion of the one or more images to the at least one imaging sensor; and at least one processor that controls one or more of the stage and the objective lens such that each portion of the one or more images is received by the at least one focusing sensor prior to it being received by the at least one imaging sensor.

In a further embodiment, a method for capturing a digital image of a slide is disclosed. The method comprises, by an objective lens having a single optical axis, acquiring one or more images of at least a portion of a sample supported on a stage; by at least one beam-splitter optically coupled to the objective lens, simultaneously providing a first portion of the one or more images to at least one focusing sensor and a second portion of the one or more images to at least one imaging sensor; and by at least one processor, controlling one or more of the stage and the objective lens such that each portion of the one or more images is received by the at least one focusing sensor prior to it being received by the at least one imaging sensor.

In an additional embodiment, a system for capturing a digital image of a slide is disclosed. The system comprises an objective lens; a stage configured to support a sample; at least one imaging sensor comprising a linear array and a wedge prism, the linear array configured to receive a light path via the objective lens; at least one processor that controls the wedge prism by moving the wedge prism into the light path during a retrace operation and moving the wedge prism out of the light path during a scan operation, receives digital image data from a retrace operation of at least a portion of the sample, determines a focus height based on the digital image data, and adjusts the height of the objective lens to the focus height prior to a scan operation of the at least a portion of the sample.

Other features and advantages of the present invention will become more readily apparent to those of ordinary skill in the art after reviewing the following detailed description and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The structure and operation of the present invention will be understood from a review of the following detailed description and the accompanying drawings in which like reference numerals refer to like parts and in which:

FIG. **1** is a block diagram illustrating an example side view configuration of a scanning system, according to an embodiment;

FIG. **2** is a block diagram illustrating an example configuration of a focusing sensor and imaging sensor with respect to a radius of illumination and a circular optical field of view, according to an embodiment;

FIG. **3**A is a block diagram illustrating an example top view configuration of a imaging sensor, according to an embodiment;

FIG. **3**B is a block diagram illustrating an example top view configuration of a focusing sensor, according to an embodiment;

FIG. **4** is a block diagram illustrating an example focusing sensor, according to an embodiment;

FIG. **5** is a time chart diagram illustrating an example interplay between a focusing sensor and an imaging sensor during scanning, according to an embodiment;

FIG. **6** is a block diagram illustrating an example non-tilted focusing sensor with a prism, according to an embodiment;

FIGS. **7**A and **7**B are block diagrams illustrating an example dual imaging and focusing sensor with a moveable prism, according to an embodiment;

FIG. **8** is a block diagram illustrating an example multiple focusing sensor configuration of a scanning system, according to an embodiment;

FIG. **9** is a block diagram illustrating an example slide motion of a scanning system, according to an embodiment;

FIG. **10** is a block diagram illustrating an example focusing sensor with a microlens array, according to an embodiment;

FIG. **11** is a block diagram illustrating an example non-tilted focusing sensor with a microlens array and a prism, according to an embodiment;

FIG. **12**A is a block diagram illustrating an example microscope slide scanner, according to an embodiment;

FIG. **12**B is a block diagram illustrating an alternative example microscope slide scanner, according to an embodiment;

FIG. **12**C is a block diagram illustrating example linear sensor arrays, according to an embodiment; and

FIG. **13** is a block diagram illustrating an example wired or wireless processor-enabled device that may be used in connection with various embodiments described herein.

DETAILED DESCRIPTION

Certain embodiments disclosed herein provide for real-time (i.e., instantaneous or near-instantaneous) focusing in line scan imaging using multiple linear detectors or other components. After reading this description it will become apparent to one skilled in the art how to implement the invention in various alternative embodiments and alternative applications. However, although various embodiments of the present invention will be described herein, it is understood that these embodiments are presented by way of example only, and not limitation. As such, this detailed description of various alternative embodiments should not be construed to limit the scope or breadth of the present invention as set forth in the appended claims.

FIG. **1** is a block diagram illustrating an example side view configuration of a scanning system **10**, according to an embodiment. In the illustrated embodiment, the scanning system **10** comprises a tissue slide **120** that is placed on a motorized stage (not shown) and illuminated by an illumination system (not shown) and moved in a scanning direction **65**. An objective lens **130** has an optical field of view that is trained on the slide **120** and provides an optical path for light from the illumination system that passes through the specimen on the slide or reflects off of the specimen on the slide or fluoresces from the specimen on the slide or otherwise passes through the objective lens **130**. The light travels on the optical path to a beam splitter **140** that allows some of the light to pass through lens **160** to an imaging sensor **20**. The light may optionally be bent by a mirror **150** as shown in the illustrated embodiment. The imaging sensor **20** may be, for example, a line charge-coupled device (CCD).

Other of the light travels from the beam splitter **140** through lens **165** to a focusing sensor **30**. The focusing sensor **30** may also be, for example, a line charge-coupled device (CCD). The light that travels to the imaging sensor **20** and the focusing sensor **30** preferably represents the complete optical field of view from the object lens **130**. Based on the configuration of the system, the scanning direction **65** of the slide **120** is logically oriented with respect to the imaging sensor **20** and the focusing sensor **30** so that the logical scanning direction **60** causes the optical field of view of the objective lens **130** to pass over the respective imaging sensor **20** and focusing sensor **20**.

As shown in the illustrated embodiment, the imaging sensor **20** is centered within the optical field of view of the objective lens **130** while the focusing sensor **30** is shifted away from the center of the optical field of view of the objective lens **130**. The direction in which the focusing sensor **30** is shifted away from the center of the optical field of view of the objective lens **130** is the opposite of the logical scanning direction **60**. This placement logically orients the focusing sensor **30** in front of the imaging sensor **20** such that as a specimen on a slide is scanned, the focusing sensor **30** "sees" the image data before the imaging sensor **20** "sees" that same image data. Finally, the focusing sensor **30** is tilted within the optical field of view such that light from the objective is sensed by the focusing sensor **30** at a plurality of Z values.

FIG. **2** is a block diagram illustrating an example configuration of a focusing sensor **30** and imaging sensor **20** with respect to a circular illumination radius **40** and an optical field of view **50**, according to an embodiment. In the illustrated embodiment, the positioning of the focusing sensor **30** is shown with respect to the imaging sensor **20** and the logical scan direction **60**. The scan direction **60** in this case, refers to the direction in which the stage or specimen (e.g., a tissue sample) is moving with respect to the sensors **20** and **30** in space. As illustrated, the sample will reach the focusing sensor **30** first, and the imaging sensor **20** second. When the imaging sensor **20** and the focusing sensor **30** are projected onto a same plane using, for example a beam-splitter, the focusing sensor **30** is within the illumination circle, which has a radius R, of the optical field of view **50** at a location ahead of the primary imaging sensor **20** in terms of the logical scanning direction **60**. Thus, when a section of a tissue sample passes the focusing sensor **30**, focus data can be captured and the focus height calculated based on one or more predetermined algorithms. The focus data and focus height can be used to control (e.g., by a controller) the distance between an objective lens height and the tissue sample before the same section of the tissue sample is sensed by the primary imaging sensor **20** via the same objective.

The circular illumination radius **40** preferably illuminates an optical field of view **50** (FOV) covering both the focusing sensor **30** and the imaging sensor **20**. The radius R is a function of the FOV on the object or sample and the optical magnification of the focusing optical path $M_{focusing}$. The function can be expressed as:

$$2R=\text{FOV}*M_{focusing}$$

For $M_{focusing}$=20 and FOV=1.325 mm (e.g., Olympus PlanApo 20× objective), R=13.25 mm. The imaging sensor **20** is projected in the middle of the optical field of view **50** for best image quality, while the focusing sensor **30** is located off-center with respect to the optical field of view **50** by a distance h from the imaging sensor **20**. There is a relationship among the distance h, the radius R, and the focusing sensor **30** length L, such that:

$$h\leq\text{square root}(R^2-(L/2)^2)$$

for a sensor length=20.48 mm and R=13.25 mm, h≤8.4 mm.

The available time t for the focusing sensor **30** to capture multiple camera lines, for focus height calculation and for moving the objective to the right Z value height, is a function of the distance h between the focusing sensor **30** and the imaging sensor **20**, magnification $M_{focusing}$, and scan speed v:

$$v*t=h/M_{focusing}$$

For a scan speed of 4.6 mm/s, the maximum time available is about 91.4 ms for $M_{focusing}$=20 and h=8.4 mm. The maximum number of camera lines captured by the focusing sensor **30**, available for the focus calculation is:

$$N=t*\kappa,$$

where □ is the line rate of the focusing sensor **30**.

For a camera line rate of 18.7 kHz, N_{max}=1,709 lines, where the objective stays at the same height. Otherwise, $N<N_{max}$ to allow the objective to move to the next focus height.

At a high level, a tissue sample is passed under an objective lens in an X direction. A portion of the tissue sample is illuminated to create an illuminated view in the Z direction of a portion of the sample. The illuminated view passes through the objective lens which is optically coupled to both the focusing sensor **30** and imaging sensor **20**, for example, using a beam splitter. The focusing sensor **30** and imaging sensor **20** are positioned such that the focusing sensor **30** receives a portion or line of the view before the imaging sensor **20** receives the same portion or line. In other words, as the focusing sensor **30** is receiving a first line of image data, the imaging sensor **20** is simultaneously receiving a second line of image data which was previously received by the focusing sensor **30** and which is a distance $h/M_{focusing}$ on the sample from the first line of image data. It will take a time period Δt for the imaging sensor **20** to receive the first line of image data after the focusing sensor **30** has received the first line of image data, where Δt represents the time that it takes the sample to move a distance $h/M_{focusing}$ in the scan direction.

During that period Δt, a processor of the scanning system **10** calculates an optimal focus height in the Z direction for the first line of image data, and adjusts the objective lens to the calculated optimal focus height. For instance, in an embodiment, the focusing sensor **30** is separate from the imaging sensor **20** and is tilted at an angle θ with respect to a direction that is perpendicular to the optical imaging path. Thus, the focusing sensor **30** receives pixels of image data at a plurality of Z values. The processor may then determine which Z height value corresponds to the pixel(s) of image data having the best focus (e.g., having the highest contrast with respect to the other pixels). After the optimal Z height value is determined, the processor or other controller may move the objective lens in the Z direction to the determined optimal Z height value before or simultaneously with the imaging sensor receiving the first line of image data.

FIG. **3**A is a block diagram illustrating an example top view configuration of a imaging sensor **20** with respect to an imaging optical path **210**, according to an embodiment. Similarly, FIG. **3**B is a block diagram illustrating an example top view configuration of a focusing sensor **30**, with respect to a focusing optical path **200**, according to an embodiment. As can be seen in FIG. **3**B, the focusing sensor **30** is tilted at an angle θ with respect to a direction that is perpendicular to the focusing optical path **200**.

FIG. **4** is a block diagram illustrating an example focusing sensor **30**, according to an embodiment. In the illustrated embodiment, within a range of focusing (z) on a tissue sample (e.g., **20** □m), the focusing sensor **30** comprises a plurality of sensor pixels **218** and may be positioned at a location where the entire focusing range (z) in the Z direction is transferred by optics to the entire focusing sensor **30** array in the Y direction (orthogonal to the X direction, i.e., scan direction), as shown. The sensor pixel **218** location is directly correlated to the Z position of the objective at focus. As illustrated in FIG. **4**, each dashed line, p_1, p_2, . . . p_i . . . p_n, across projected focusing range (d) represents a different focus value and corresponds to a focus height, i.e., Z height, of the objective lens. The p_i having the optimal focus for a given portion of a sample can be used by the scanning system to determine the optimal focus height for that portion of the sample.

The relationship between the projected focusing range (d) on the focusing sensor **30** and the focusing range (z) on the specimen object is as follows:

$$d=z*M_{focusing}^2,$$

where $M_{focusing}$ is the optical magnification of the focusing path. For instance, if z=20 □m and $M_{focusing}$=20, then d=8 mm.

In order to cover the entire projected focusing range (d) by a tilted focusing sensor **30** that is a linear array sensor, the tilting angle □ should follow the relationship:

$$\sin \square = d/L,$$

where *L* is the length of the sensor **30**.

Using d=8 mm and L=20.48 mm, □=23.0°. □□ and L can vary as long as the tilted sensor **30** covers the entire focusing range (z).

The focusing resolution, or the minimum step of objective height motion □z is a function of the sensor pixel size, e=minimum (□L). Derived from the above formulas:

$$\square z = e*z/L.$$

For instance, if e=10 □m, L=20.48 mm, and z=20 □m, then □z=0.0097 □m<10 nm.

In an embodiment, one-dimensional data from the focusing sensor **30** is analyzed. A figure of merit (FOM) (e.g., contrast of the data) may be defined. The pixel **218** location (Z value) of the maximum FOM on the sensor array can be found. In this manner, the focus position (Z value) of the objective can be determined for that scan line on the sample.

The relationship between the objective height Z_i and the focus location L_i on the focusing sensor of focus point i is:

$$L_i = Z_i * M_{focusing}^2 / \sin \square$$

If the focus height is determined by a mean from L_1 to L_2, according to the analysis of the data from the focusing sensor **30** discussed above, the objective height needs to be moved from Z_1 to Z_2 based on:

$$Z_2 = Z_1 + (L_2 - L_1) * \sin \square / M_{focusing}^2$$

Although the field of view (FOV) in the Y axis of the focusing sensor **30** and the imaging sensor **20** can be different, the centers of both sensors are preferably aligned to each other along the Y axis.

FIG. **5** is a time chart diagram illustrating an example interplay between a focusing sensor **30** and an imaging sensor **20** during scanning, according to an embodiment. In the illustrated embodiment, the timing of a scan using an imaging sensor **30** and focusing sensor **20** is shown. At time t_0, the Z position of the objective lens is at height Z_0 on tissue section X_1, which is in the field of view of the focusing sensor **30**. The focusing sensor **30** receives focusing data corresponding to tissue section X_1. The focus height Z_1 is determined to be the optimal focus height for tissue section X_1 using the focusing data and, in some embodiments, associated focusing algorithms. The optimal focus height is then fed to the Z positioner to move the objective lens to the height Z_1, for example, using a control loop. At t_1, tissue section X_1 is moved into the field of view of the imaging sensor **20**. With the correct focus height, the imaging sensor **20** will sense an optimally focused image of the sample. At the same time t_1, the focusing sensor **30** captures focusing data from tissue section X_2, and the focusing data will be used to determine the optimal focus height Z_2 which in turn will be fed into the Z positioner prior to or at the time that tissue section X_2 passes into the field of view of the imaging sensor **20** at time t_2. Such a process can continue until the entire tissue sample is scanned.

In general at time t_n, tissue section X_{n+1} is in the field of view of the focusing sensor, tissue section X_n is in the field of view of the imaging sensor **30**, and the objective lens is at a Z height of Z_n. Furthermore, prior to or at the time t_{n+1}, the optimal focus height for tissue section X_{n+1} is determined and the Z height of the objective lens is adjusted to Z_{n+1}. Considering FIG. **2**, at time t_0, the focusing sensor **30** "sees" tissue section X_1 and determines the focus height as Z_1 for tissue section X_1; at time t_1, tissue section X_1 moves under the imaging sensor **20** and the objective moves to height Z_1 while the focusing sensor **30** "sees" tissue section X_2 and determines the focus height as Z_2 for tissue section X_2; at time t_n, tissue section X_n moves under the imaging sensor **20** and the objective moves to height Z_n while the focusing sensor **30** "sees" tissue section X_{n+1} and determines the focus height as Z_{n+1} for tissue section X_{n+1}. A person of skill in the art should understand that X_{n-1} and X_n do not necessarily represent consecutive or adjacent lines of image data, as long as a scan line is acquired by the focusing sensor **30** and an optimal focus height for the scan line is determined and set prior to the same scan line being acquired by the imaging sensor **20**. In other words, the focusing sensor **30** and imaging sensor **20** may be arranged such that one or more scan lines exist between the focusing sensor's **30** field of view and the imaging sensor's **20** field of view, i.e., that distance h between the focusing sensor **30** and imaging sensor **20** comprises one or more scan lines of data. For instance, in that case that the distance h comprises 5 scan lines, tissue section X_6 would be in the field of view of the focusing sensor **30** at the same time that tissue section X_1 is in the field of view of the imaging sensor **20**. In this case, the focus height of the objective lens would be adjusted to the calculated optimal focus height after the tissue section X_5 is sensed by the imaging sensor **20** but prior to the tissue section X_6 being sensed by the imaging sensor **20**. Advantageously, the focus height of the objective lens may be smoothly controlled between tissue section X_1 and X_6 such that there are incremental changes in focus height between X_1 and X_6 that approximate a gradual slope of the tissue sample.

FIG. **6** is a block diagram illustrating an example non-tilted focusing sensor **30** with a prism **270**, according to an embodiment. In the illustrated embodiment, FIG. **6** shows an alternative to the tilted focusing line sensor **30**. Instead of tilting the focusing sensor **30**, a wedge prism **270** attached to the focusing sensor **30** can be used to perform a similar focusing function. Alternatively, a combination of a wedge prism **270** and a tilted focusing sensor **30** could be employed. The algorithms and procedure may remain the same in such an alternative embodiment. Furthermore, if two parallel linear sensor arrays with sufficient spacing between the sensors can be integrated into a single field of view, a wedge prism **270** can be attached onto the focusing sensor **30** for focusing and the imaging sensor **20** can be used for sensing image data from the sample.

FIGS. **7**A and **7**B are block diagrams illustrating an example dual imaging and focusing sensor with a moveable prism, according to an embodiment. Advantageously, in this embodiment the same physical sensor can be used as both the focusing sensor **30** and the imaging sensor **20**. As shown in FIG. **7**A, when the wedge prism **270** is in place, the sensor performs the functions of the focusing sensor **30**. The wedge prism **270** is in place when the wedge prism **270** is placed within the field of view of at least a portion of the sensor. Correspondingly, when the wedge prism **270** is not in place, the sensor performs the functions of the imaging sensor **20**. The wedge prism **270** is not in place when no portion of the wedge prism **270** is within the field of view of the sensor.

Such an embodiment can be used in connection with a scanning motion such as the motion described with respect to FIG. **9** whereby the scanning system focuses during a first pass of the sample under the objective lens and then the scanning system images during a second pass of the same portion of the sample under the objective lens. During the first pass, the wedge prism **270** is in place and during the second pass, the wedge prism **270** is not in place.

FIG. **8** is a block diagram illustrating an example multiple focusing sensor configuration of a scanning system, according to an embodiment. In the illustrated embodiment, the scanning system employs at least two focusing sensors **30** and **35**. In the illustrated embodiment, the second focusing sensor **35** may comprise a linear sensory array tilted in the opposite direction as the first focusing sensor **30**. The second focusing sensor **35** can perform the same focusing function as the first focusing sensor **30** to allow the scanning system to analyze data obtained from the two focusing sensors **30** and **35** and calculate a more precise optimal focus height for the objective lens when the imaging sensor passes over the specimen. For example, the scanning system may use the dual focusing sensor **30** **35** data for averaging or compensation. The second focusing sensor **35** may be the third linear sensor array of the scanning system, when combined with the first focusing sensor **30** and the primary imaging sensor **20**.

FIG. **10** is a block diagram illustrating an example focusing sensor **30** with a microlens array **250**, according to an embodiment. In the illustrated embodiment, a linear microlens array **250** (tilted or non-tilted) is positioned in the FOV of the tilted focusing sensor **30** (e.g., a linear sensor array) to have multiple micro images of an identical tissue section. This embodiment can be used to avoid possible ambiguity associated with partial tissue within the FOV, which may result from previously described embodiments and methods. In one embodiment, the minimum dimension of the microlens array **250** is to cover the cross-section of the light path falling onto the focusing sensor **30**, whether the microlens array **250** is either tilted or not tilted. The number of microlens array **250** elements is determined by the Z resolution and the total focusing range. For instance, if a 0.5 □m resolution is required over a 20 □m focusing range, the number of lens elements is 40.

In one embodiment, the field lens **260** is used to reduce the vignetting effect in the scanning system and does not impact system magnification. In a microlens array **250** embodiment, the method to determine the best focus may be different than the figure of merit technique described above. For example, the scanning system may compare the average contrast, for example, among 40 microlens images with a 0.5 □m depth increment. The center location of the highest contrast in the image is found and the objective height is then determined based on the same formula as discussed above: $L_i = Z_i * M_{focusing}^2 / \sin \square$.

FIG. **11** is a block diagram illustrating an example non-tilted focusing sensor **30** with a microlens array **250** and a prism **270**, according to an embodiment. In the illustrated embodiment, the microlens array **250** is integrated with a wedge prism **270** in front of the focusing sensor **30** (e.g., a line sensor) to perform the focusing function.

FIGS. **12**A and **12**B are block diagrams illustrating example microscope slide scanners, according to an embodiment and FIG. **12**C is a block diagram illustrating example linear sensor arrays, according to an embodiment. These three figures will be described in more detail below, however

they will first be described in combination to provide an overview. It should be noted that the following description is just an example of a slide scanner device and that alternative slide scanner devices can also be employed. FIGS. **12**A and **12**B illustrate example microscope slide scanners that can be used in conjunction with the disclosed sensor arrangement. FIG. **12**C illustrates example linear sensors, which can be used in any combination as the disclosed sensors (imaging sensors or focusing sensors).

For example, the imaging sensor and the focusing sensor(s) may be arranged (e.g., in conjunction with a beam-splitter), as discussed above, using line scan camera **18** as the primary imaging sensor and focusing sensor **20** as the focusing sensor in combination with the beam splitter **140**. In one embodiment, the line scan camera **18** may include both the focusing sensor and the primary imaging sensor. The imaging sensor and focusing sensor(s) can receive image information from a sample **12** through the microscope objective lens **16** and/or the focusing optics **34** and **290**. Furthermore, they can provide information to, and/or receive information from, data processor **21**. Data processor **21** is communicatively connected to memory **36** and data storage **38**. Data processor **21** may further be communicatively connected to a communications port, which may be connected by at least one network **42** to one or more computers **44**, which may in turn be connected to display monitor(s) **46**.

Data processor **21** may also be communicatively connected to and provide instructions to a stage controller **22**, which controls a motorized stage **14** of the slide scanner **11**. The motorized stage **14** supports sample **12** and moves in one or more directions in the X-Y plane. In one embodiment, the motorized stage **14** may also move in the Z plane. Data processor **21** may also be communicatively connected to and provide instructions to a motorized controller **26**, which controls a motorized positioner **24** (e.g., a piezo positioner). The motorized positioner **24** is configured to move the objective lens **16** in the Z direction. The slide scanner **11** also comprises a light source **31** and/or illumination optics **32** to illuminate the sample **12**, either from above or below.

Turning now to FIG. **12**A, a block diagram of an embodiment of an optical microscopy system **10** according to the present invention is shown. The heart of the system **10** is a microscope slide scanner **11** that serves to scan and digitize a specimen or sample **12**. The sample **12** can be anything that may be interrogated by optical microscopy. For instance, the sample **12** may be a microscope slide or other sample type that may be interrogated by optical microscopy. A microscope slide is frequently used as a viewing substrate for specimens that include tissues and cells, chromosomes, DNA, protein, blood, bone marrow, urine, bacteria, beads, biopsy materials, or any other type of biological material or substance that is either dead or alive, stained or unstained, labeled or unlabeled. The sample **12** may also be an array of any type of DNA or DNA-related material such as cDNA or RNA or protein that is deposited on any type of slide or other substrate, including any and all samples commonly known as a microarrays. The sample **12** may be a microtiter plate, for example a 96-well plate. Other examples of the sample **12** include integrated circuit boards, electrophoresis records, petri dishes, film, semiconductor materials, forensic materials, or machined parts.

The scanner **11** includes a motorized stage **14**, a microscope objective lens **16**, a line scan camera **18**, and a data processor **21**. The sample **12** is positioned on the motorized stage **14** for scanning. The motorized stage **14** is connected to a stage controller **22** which is connected in turn to the data processor **21**. The data processor **21** determines the position of the sample **12** on the motorized stage **14** via the stage controller **22**. In one embodiment, the motorized stage **14** moves the sample **12** in at least the two axes (x/y) that are in the plane of the sample **12**. Fine movements of the sample **12** along the optical z-axis may also be necessary for certain applications of the scanner **11**, for example, for focus control. Z-axis movement is preferably accomplished with a piezo positioner **24**, such as the PIFOC from Polytec PI or the MIPOS 3 from Piezosystem Jena. The piezo positioner **24** is attached directly to the microscope objective **16** and is connected to and directed by the data processor **21** via a piezo controller **26**. A means of providing a coarse focus adjustment may also be needed and can be provided by z-axis movement as part of the motorized stage **14** or a manual rack-and-pinion coarse focus adjustment (not shown).

In one embodiment, the motorized stage **14** includes a high precision positioning table with ball bearing linear ways to provide smooth motion and excellent straight line and flatness accuracy. For example, the motorized stage **14** could include two Daedal model 106004 tables stacked one on top of the other. Other types of motorized stages **14** are also suitable for the scanner **11**, including stacked single axis stages based on ways other than ball bearings, single- or multiple-axis positioning stages that are open in the center and are particularly suitable for trans-illumination from below the sample, or larger stages that can support a plurality of samples. In one embodiment, motorized stage **14** includes two stacked single-axis positioning tables, each coupled to two millimeter lead-screws and Nema-23 stepping motors. At the maximum lead screw speed of twenty-five revolutions per second, the maximum speed of the sample **12** on the motorized stage **14** is fifty millimeters per second. Selection of a lead screw with larger diameter, for example five millimeters, can increase the maximum speed to more than 100 millimeters per second. The motorized stage **14** can be equipped with mechanical or optical position encoders which has the disadvantage of adding significant expense to the system. Consequently, such an embodiment does not include position encoders. However, if one were to use servo motors in place of stepping motors, then one would have to use position feedback for proper control.

Position commands from the data processor **21** are converted to motor current or voltage commands in the stage controller **22**. In one embodiment, the stage controller **22** includes a 2-axis servo/stepper motor controller (Compumotor 6K2) and two 4-amp microstepping drives (Compumotor OEMZL4). Microstepping provides a means for commanding the stepper motor in much smaller increments than the relatively large single 1.8 degree motor step. For example, at a microstep of 100, the sample **12** can be commanded to move at steps as small as 0.1 micrometer. A microstep of 25,000 is used in one embodiment of this invention. Smaller step sizes are also possible. It should be obvious that the optimum selection of the motorized stage **14** and the stage controller **22** depends on many factors, including the nature of the sample **12**, the desired time for sample digitization, and the desired resolution of the resulting digital image of the sample **12**.

The microscope objective lens **16** can be any microscope objective lens commonly available. One of ordinary skill in the art will realize that the choice of which objective lens to use will depend on the particular circumstances. In one embodiment of the present invention, the microscope objective lens **16** is of the infinity-corrected type.

The sample **12** is illuminated by an illumination system **28** that includes a light source **31** and illumination optics **32**.

The light source **31** in one embodiment includes a variable intensity halogen light source with a concave reflective mirror to maximize light output and a KG-1 filter to suppress heat. However, the light source **31** could also be any other type of arc-lamp, laser, light emitting diode ("LED") or other source of light. The illumination optics **32** in one embodiment include a standard Köhler illumination system with two conjugate planes that are orthogonal to the optical axis. The illumination optics **32** are representative of the bright-field illumination optics that can be found on most commercially available compound microscopes sold by companies such as Carl Zeiss, Nikon, Olympus, or Leica. One set of conjugate planes includes (i) a field iris aperture illuminated by the light source **31**, (ii) the object plane that is defined by the focal plane of the sample **12**, and (iii) the plane containing the light-responsive elements of the line scan camera **18**. A second conjugate plane includes (i) the filament of the bulb that is part of the light source **31**, (ii) the aperture of a condenser iris that sits immediately before the condenser optics that are part of the illumination optics **32**, and (iii) the back focal plane of the microscope objective lens **16**. In one embodiment, the sample **12** is illuminated and imaged in transmission mode, with the line scan camera **18** sensing optical energy that is transmitted by the sample **12**, or conversely, optical energy that is absorbed by the sample **12**.

The scanner **11** of the present invention is equally suitable for detecting optical energy that is reflected from the sample **12**, in which case the light source **31**, the illumination optics **32**, and the microscope objective lens **16** must be selected based on compatibility with reflection imaging. One possible embodiment may therefore be illumination through a fiber optic bundle that is positioned above the sample **12**. Other possibilities include excitation that is spectrally conditioned by a monochromator. If the microscope objective lens **16** is selected to be compatible with phase-contrast microscopy, then the incorporation of at least one phase stop in the condenser optics that are part of the illumination optics **32** will enable the scanner **11** to be used for phase contrast microscopy. To one of ordinary skill in the art, the modifications required for other types of microscopy such as differential interference contrast and confocal microscopy should be readily apparent. Overall, the scanner **11** is suitable, with appropriate but well-known modifications, for the interrogation of microscopic samples in any known mode of optical microscopy.

Between the microscope objective lens **16** and the line scan camera **18** are situated the line scan camera focusing optics **34** that focus the optical signal captured by the microscope objective lens **16** onto the light-responsive elements of the line scan camera **18**. In a modern infinity-corrected microscope the focusing optics between the microscope objective lens and the eyepiece optics, or between the microscope objective lens and an external imaging port, consist of an optical element known as a tube lens that is part of a microscope's observation tube. Many times the tube lens consists of multiple optical elements to prevent the introduction of coma or astigmatism. One of the motivations for the relatively recent change from traditional finite tube length optics to infinity corrected optics was to increase the physical space in which the optical energy from the sample **12** is parallel, meaning that the focal point of this optical energy is at infinity. In this case, accessory elements like dichroic minors or filters can be inserted into the infinity space without changing the optical path magnification or introducing undesirable optical artifacts.

Infinity-corrected microscope objective lenses are typically inscribed with an infinity mark. The magnification of an infinity corrected microscope objective lens is given by the quotient of the focal length of the tube lens divided by the focal length of the objective lens. For example, a tube lens with a focal length of 180 millimeters will result in 20× magnification if an objective lens with 9 millimeter focal length is used. One of the reasons that the objective lenses manufactured by different microscope manufacturers are not compatible is because of a lack of standardization in the tube lens focal length. For example, a 20× objective lens from Olympus, a company that uses a 180 millimeter tube lens focal length, will not provide a 20× magnification on a Nikon microscope that is based on a different tube length focal length of 200 millimeters. Instead, the effective magnification of such an Olympus objective lens engraved with 20× and having a 9 millimeter focal length will be 22.2×, obtained by dividing the 200 millimeter tube lens focal length by the 9 millimeter focal length of the objective lens. Changing the tube lens on a conventional microscope is virtually impossible without disassembling the microscope. The tube lens is part of a critical fixed element of the microscope. Another contributing factor to the incompatibility between the objective lenses and microscopes manufactured by different manufacturers is the design of the eyepiece optics, the binoculars through which the specimen is observed. While most of the optical corrections have been designed into the microscope objective lens, most microscope users remain convinced that there is some benefit in matching one manufacturers' binocular optics with that same manufacturers' microscope objective lenses to achieve the best visual image.

The line scan camera focusing optics **34** include a tube lens optic mounted inside of a mechanical tube. Since the scanner **11**, in one embodiment, lacks binoculars or eyepieces for traditional visual observation, the problem suffered by conventional microscopes of potential incompatibility between objective lenses and binoculars is immediately eliminated. One of ordinary skill will similarly realize that the problem of achieving parfocality between the eyepieces of the microscope and a digital image on a display monitor is also eliminated by virtue of not having any eyepieces. Since the scanner **11** also overcomes the field of view limitation of a traditional microscope by providing a field of view that is practically limited only by the physical boundaries of the sample **12**, the importance of magnification in an all-digital imaging microscope such as provided by the present scanner **11** is limited. Once a portion of the sample **12** has been digitized, it is straightforward to apply electronic magnification, sometimes known as electric zoom, to an image of the sample **12** in order to increase its magnification. Increasing the magnification of an image electronically has the effect of increasing the size of that image on the monitor that is used to display the image. If too much electronic zoom is applied, then the display monitor will be able to show only portions of the magnified image. It is not possible, however, to use electronic magnification to display information that was not present in the original optical signal that was digitized in the first place. Since one of the objectives of the scanner **11** is to provide high quality digital images, in lieu of visual observation through the eyepieces of a microscope, it is important that the content of the images acquired by the scanner **11** include as much image detail as possible. The term resolution is typically used to describe such image detail and the term diffraction-limited is used to describe the wavelength-limited maximum spatial detail available in an optical signal. The scanner **11**

provides diffraction-limited digital imaging by selection of a tube lens focal length that is matched according to the well know Nyquist sampling criteria to both the size of an individual pixel element in a light-sensing camera such as the line scan camera **18** and to the numerical aperture of the microscope objective lens **16**. It is well known that numerical aperture, not magnification, is the resolution-limiting attribute of a microscope objective lens **16**.

An example will help to illustrate the optimum selection of a tube lens focal length that is part of the line scan camera focusing optics **34**. Consider again the 20× microscope objective lens **16** with 9 millimeter focal length discussed previously and assume that this objective lens has a numerical aperture of 0.50. Assuming no appreciable degradation from the condenser, the diffraction-limited resolving power of this objective lens at a wavelength of 500 nanometers is approximately 0.6 micrometers, obtained using the well-known Abbe relationship. Assume further that the line scan camera **18**, which in one embodiment has a plurality of 14 micrometer square pixels, is used to detect a portion of the sample **12**. In accordance with sampling theory, it is necessary that at least two sensor pixels subtend the smallest resolvable spatial feature. In this case, the tube lens must be selected to achieve a magnification of 46.7, obtained by dividing 28 micrometers, which corresponds to two 14 micrometer pixels, by 0.6 micrometers, the smallest resolvable feature dimension. The optimum tube lens optic focal length is therefore about 420 millimeters, obtained by multiplying 46.7 by 9. The line scan focusing optics **34** with a tube lens optic having a focal length of 420 millimeters will therefore be capable of acquiring images with the best possible spatial resolution, similar to what would be observed by viewing a specimen under a microscope using the same 20× objective lens. To reiterate, the scanner **11** utilizes a traditional 20× microscope objective lens **16** in a higher magnification optical configuration, in this example about 47×, in order to acquire diffraction-limited digital images. If a traditional 20× magnification objective lens **16** with a higher numerical aperture were used, say 0.75, the required tube lens optic magnification for diffraction-limited imaging would be about 615 millimeters, corresponding to an overall optical magnification of 68×. Similarly, if the numerical aperture of the 20× objective lens were only 0.3, the optimum tube lens optic magnification would only be about 28×, which corresponds to a tube lens optic focal length of approximately 252 millimeters. The line scan camera focusing optics **34** are modular elements of the scanner **11** and can be interchanged as necessary for optimum digital imaging. The advantage of diffraction-limited digital imaging is particularly significant for applications, for example bright field microscopy, in which the reduction in signal brightness that accompanies increases in magnification is readily compensated by increasing the intensity of an appropriately designed illumination system **28**.

In principle, it is possible to attach external magnification-increasing optics to a conventional microscope-based digital imaging system to effectively increase the tube lens magnification so as to achieve diffraction-limited imaging as has just been described for the present scanner **11**; however, the resulting decrease in the field of view is often unacceptable, making this approach impractical. Furthermore, many users of microscopes typically do not understand enough about the details of diffraction-limited imaging to effectively employ these techniques on their own. In practice, digital cameras are attached to microscope ports with magnification-decreasing optical couplers to attempt to increase the size of the field of view to something more similar to what can be seen through the eyepiece. The standard practice of adding de-magnifying optics is a step in the wrong direction if the goal is to obtain diffraction-limited digital images.

In a conventional microscope, different power objectives lenses are typically used to view the specimen at different resolutions and magnifications. Standard microscopes have a nosepiece that holds five objectives lenses. In an all-digital imaging system such as the present scanner **11** there is a need for only one microscope objective lens **16** with a numerical aperture corresponding to the highest spatial resolution desirable. One embodiment of the scanner **11** provides for only one microscope objective lens **16**. Once a diffraction-limited digital image has been captured at this resolution, it is straightforward using standard digital image processing techniques, to present imagery information at any desirable reduced resolutions and magnifications.

One embodiment of the scanner **11** is based on a Dalsa SPARK line scan camera **18** with 1024 pixels (picture elements) arranged in a linear array, with each pixel having a dimension of 14 by 14 micrometers. Any other type of linear array, whether packaged as part of a camera or custom-integrated into an imaging electronic module, can also be used. The linear array in one embodiment effectively provides eight bits of quantization, but other arrays providing higher or lower level of quantization may also be used. Alternate arrays based on 3-channel red-green-blue (RGB) color information or time delay integration (TDI), may also be used. TDI arrays provide a substantially better signal-to-noise ratio (SNR) in the output signal by summing intensity data from previously imaged regions of a specimen, yielding an increase in the SNR that is in proportion to the square-root of the number of integration stages. TDI arrays can comprise multiple stages of linear arrays. TDI arrays are available with 24, 32, 48, 64, 96, or even more stages. The scanner **11** also supports linear arrays that are manufactured in a variety of formats including some with 512 pixels, some with 1024 pixels, and others having as many as 4096 pixels. Appropriate, but well known, modifications to the illumination system **28** and the line scan camera focusing optics **34** may be required to accommodate larger arrays. Linear arrays with a variety of pixel sizes can also be used in scanner **11**. The salient requirement for the selection of any type of line scan camera **18** is that the sample **12** can be in motion with respect to the line scan camera **18** during the digitization of the sample **12** in order to obtain high quality images, overcoming the static requirements of the conventional imaging tiling approaches known in the prior art.

The output signal of the line scan camera **18** is connected to the data processor **21**. The data processor **21** in one embodiment includes a central processing unit with ancillary electronics, for example a motherboard, to support at least one signal digitizing electronics board such as an imaging board or a frame grabber. In the presently embodiment, the imaging board is an EPIX PIXCID24 PCI bus imaging board, however, there are many other types of imaging boards or frame grabbers from a variety of manufacturers which could be used in place of the EPIX board. An alternate embodiment could be a line scan camera that uses an interface such as IEEE 1394, also known as Firewire, to bypass the imaging board altogether and store data directly on a data storage **38**, such as a hard disk.

The data processor **21** is also connected to a memory **36**, such as random access memory (RAM), for the short-term storage of data, and to the data storage **38**, such as a hard drive, for long-term data storage. Further, the data processor **21** is connected to a communications port **40** that is connected to a network **42** such as a local area network (LAN),

a wide area network (WAN), a metropolitan area network (MAN), an intranet, an extranet, or the global Internet. The memory **36** and the data storage **38** are also connected to each other. The data processor **21** is also capable of executing computer programs, in the form of software, to control critical elements of the scanner **11** such as the line scan camera **18** and the stage controller **22**, or for a variety of image-processing functions, image-analysis functions, or networking. The data processor **21** can be based on any operating system, including operating systems such as Windows, Linux, OS/2, Mac OS, and Unix. In one embodiment, the data processor **21** operates based on the Windows NT operating system.

The data processor **21**, memory **36**, data storage **38**, and communication port **40** are each elements that can be found in a conventional computer. One example would be a personal computer such as a Dell Dimension XPS T500 that features a Pentium III 500 MHz processor and up to 756 megabytes (MB) of RAM. In one embodiment, the computer, elements which include the data processor **21**, memory **36**, data storage **38**, and communications port **40** are all internal to the scanner **11**, so that the only connection of the scanner **11** to the other elements of the system **10** is the communication port **40**. In an alternate embodiment of the scanner **11**, the computer elements would be external to the scanner **11** with a corresponding connection between the computer elements and the scanner **11**.

The scanner **11**, in one embodiment of the invention, integrates optical microscopy, digital imaging, motorized sample positioning, computing, and network-based communications into a single-enclosure unit. The major advantage of packaging the scanner **11** as a single-enclosure unit with the communications port **40** as the primary means of data input and output are reduced complexity and increased reliability. The various elements of the scanner **11** are optimized to work together, in sharp contrast to traditional microscope-based imaging systems in which the microscope, light source, motorized stage, camera, and computer are typically provided by different vendors and require substantial integration and maintenance.

The communication port **40** provides a means for rapid communications with the other elements of the system **10**, including the network **42**. One communications protocol for the communications port **40** is a carrier-sense multiple-access collision detection protocol such as Ethernet, together with the TCP/IP protocol for transmission control and internetworking. The scanner **11** is intended to work with any type of transmission media, including broadband, baseband, coaxial cable, twisted pair, fiber optics, DSL or wireless.

In one embodiment, control of the scanner **11** and review of the imagery data captured by the scanner **11** are performed on a computer **44** that is connected to the network **42**. The computer **44**, in one embodiment, is connected to a display monitor **46** to provide imagery information to an operator. A plurality of computers **44** may be connected to the network **42**. In one embodiment, the computer **44** communicates with the scanner **11** using a network browser such as Internet Explorer from Microsoft or Netscape Communicator from AOL. Images are stored on the scanner **11** in a common compressed format such a JPEG which is an image format that is compatible with standard image-decompression methods that are already built into most commercial browsers. Other standard or non-standard, lossy or lossless, image compression formats will also work. In one embodiment, the scanner **11** is a webserver providing an operator interface that is based on webpages that are sent from the scanner **11** to the computer **44**. For dynamic review of imagery data, one embodiment of the scanner **11** is based on playing back, for review on the display monitor **46** that is connected to the computer **44**, multiple frames of imagery data using standard multiple-frame browser compatible software packages such as Media-Player from Microsoft, Quicktime from Apple Computer, or RealPlayer from Real Networks. In one embodiment, the browser on the computer **44** uses the hypertext transmission protocol (http) together with TCP for transmission control.

There are, and will be in the future, many different means and protocols by which the scanner **11** could communicate with the computer **44**, or a plurality of computers. While one embodiment is based on standard means and protocols, the approach of developing one or multiple customized software modules known as applets is equally feasible and may be desirable for selected future applications of the scanner **11**. Further, there are no constraints that computer **44** be of any specific type such as a personal computer (PC) or be manufactured by any specific company such as Dell. One of the advantages of a standardized communications port **40** is that any type of computer **44** operating common network browser software can communicate with the scanner **11**.

If one so desires, it is possible, with some modifications to the scanner **11**, to obtain spectrally resolved images. Spectrally resolved images are images in which spectral information is measured at every image pixel. Spectrally resolved images could be obtained by replacing the line scan camera **18** of the scanner **11** with an optical slit and an imaging spectrograph. The imaging spectrograph uses a two-dimensional CCD detector to capture wavelength-specific intensity data for a column of image pixels by using a prism or grating to disperse the optical signal that is focused on the optical slit along each of the rows of the detector.

Turning now to FIG. **12**B, a block diagram of a second embodiment of an optical microscopy system **10** according to the present invention is shown. In this system **10**, the scanner **11** is more complex and expensive than the embodiment shown in FIG. **12**A. The additional attributes of the scanner **11** that are shown do not all have to be present for any alternate embodiment to function correctly. FIG. **2** is intended to provide a reasonable example of additional features and capabilities that could be incorporated into the scanner **11**.

The alternate embodiment of FIG. **12**B provides for a much greater level of automation than the embodiment of FIG. **12**A. A more complete level of automation of the illumination system **28** is achieved by connections between the data processor **21** and both the light source **31** and the illumination optics **32** of the illumination system **28**. The connection to the light source **31** may control the voltage, or current, in an open or closed loop fashion, in order to control the intensity of the light source **31**. Recall that the light source **31** is a halogen bulb in one embodiment. The connection between the data processor **21** and the illumination optics **32** could provide closed loop control of the field iris aperture and the condenser iris to provide a means for ensuring that optimum Kohler illumination is maintained.

Use of the scanner **11** for fluorescence imaging requires easily recognized modifications to the light source **31**, the illumination optics **32**, and the microscope objective lens **16**. The second embodiment of FIG. **12**B also provides for a fluorescence filter cube **50** that includes an excitation filter, a dichroic filter, and a barrier filter. The fluorescence filter cube **50** is positioned in the infinity corrected beam path that exists between the microscope objective lens **16** and line scan camera focusing optics **34**. One embodiment for fluorescence imaging could include the addition of a filter wheel

or tunable filter into the illumination optics **32** to provide appropriate spectral excitation for the variety of fluorescent dyes or nano-crystals available on the market.

The addition of at least one beam splitter **52** into the imaging path allows the optical signal to be split into at least two paths. The primary path is via the line scan camera focusing optics **34**, as discussed previously, to enable diffraction-limited imaging by the line scan camera **18**. A second path is provided via an area scan camera focusing optics **54** for imaging by an area scan camera **56**. It should be readily apparent that proper selection of these two focusing optics can ensure diffraction-limited imaging by the two camera sensors having different pixel sizes. The area scan camera **56** can be one of many types that are currently available, including a simple color video camera, a high performance, cooled, CCD camera, or a variable integration-time fast frame camera. The area scan camera **56** provides a traditional imaging system configuration for the scanner **11**. The area scan camera **56** is connected to the data processor **21**. If two cameras are used, for example the line scan camera **18** and the area scan camera **56**, both camera types could be connected to the data processor using either a single dual-purpose imaging board, two different imaging boards, or the IEEE1394 Firewire interface, in which case one or both imaging boards may not be needed. Other related methods of interfacing imaging sensors to the data processor **21** are also available.

While the primary interface of the scanner **11** to the computer **44** is via the network **42**, there may be instances, for example a failure of the network **42**, where it is beneficial to be able to connect the scanner **11** directly to a local output device such as a display monitor **58** and to also provide local input devices such as a keyboard and mouse **60** that are connected directly into the data processor **21** of the scanner **11**. In this instance, the appropriate driver software and hardware would have to be provided as well.

The second embodiment shown in FIG. **12**B also provides for a much greater level of automated imaging performance. Enhanced automation of the imaging of the scanner **11** can be achieved by closing the focus control loop comprising the piezo positioner **24**, the piezo controller **26**, and the data processor **21** using well-known methods of autofocus. The second embodiment also provides for a motorized nose-piece **62** to accommodate several objectives lenses. The motorized nose-piece **62** is connected to and directed by the data processor **21** through a nose-piece controller **64**.

There are other features and capabilities of the scanner **11** which could be incorporated. For example, the process of scanning the sample **12** with respect to the microscope objective lens **16** that is substantially stationary in the x/y plane of the sample **12** could be modified to comprise scanning of the microscope objective lens **16** with respect to a stationary sample **12**. Scanning the sample **12**, or scanning the microscope objective lens **16**, or scanning both the sample **12** and the microscope objective lens **16** simultaneously, are possible embodiments of the scanner **11** which can provide the same large contiguous digital image of the sample **12** as discussed previously.

The scanner **11** also provides a general purpose platform for automating many types of microscope-based analyses. The illumination system **28** could be modified from a traditional halogen lamp or arc-lamp to a laser-based illumination system to permit scanning of the sample **12** with laser excitation. Modifications, including the incorporation of a photomultiplier tube or other non-imaging detector, in addition to or in lieu of the line scan camera **18** or the area scan camera **56**, could be used to provide a means of detecting the optical signal resulting from the interaction of the laser energy with the sample **12**.

Turning now to FIG. **12**C, the line scan camera field of view **70** comprises the region of the sample **12** of FIG. **12**A that is imaged by a multitude of individual pixel elements **72** that are arranged in a linear fashion into a linear array **74** as shown in FIG. **12**C. The linear array **74** of one embodiment comprises 1024 of the individual pixel elements **72**, with each of the pixel elements **72** being 14 micrometers square. The physical dimensions of the linear array **74** of one embodiment are 14.34 millimeters by 14 micrometers. Assuming, for purposes of discussion of the operation of the scanner **11**, that the magnification between the sample **12** and the line scan camera **18** is ten, then the line scan camera field of view **70** corresponds to a region of the sample **12** that has dimensions equal to 1.43 millimeters by 1.4 micrometers. Each pixel element **72** images an area about 1.4 micrometers by 1.4 micrometers.

In one embodiment of the scanner **11**, the scanning and digitization is performed in a direction of travel **84** that alternates between image strips. This type of bi-directional scanning provides for a more rapid digitization process than uni-directional scanning, a method of scanning and digitization which requires the same direction of travel **84** for each image strip.

The capabilities of the line scan camera **18** and the focusing sensor **30** typically determine whether scanning and focusing can be done bi-directionally or uni-directionally. Uni-directional systems often comprise more than one linear array **74**, such as a three channel color array **86** or a multi-channel TDI array **88** shown in FIG. **12**C. The color array **86** detects the RGB intensities required for obtaining a color image. An alternate embodiment for obtaining color information uses a prism to split the broadband optical signal into the three color channels. The TDI array **88** could be used in an alternate embodiment of the scanner **11** to provide a means of increasing the effective integration time of the line scan camera **18**, while maintaining a fast data rate, and without significant loss in the signal-to-noise ratio of the digital imagery data.

FIG. **13** is a block diagram illustrating an example wired or wireless processor-enabled device **550** that may be used in connection with various embodiments described herein. For example the system **550** may be used as or in conjunction with the line scanning system as previously described. For example, the system **550** can be used to control the various elements of the line scanning system. The system **550** can be a conventional personal computer, computer server, personal digital assistant, smart phone, tablet computer, or any other processor enabled device that is capable of wired or wireless data communication. Other computer systems and/or architectures may be also used, as will be clear to those skilled in the art.

The system **550** preferably includes one or more processors, such as processor **560**. Additional processors may be provided, such as an auxiliary processor to manage input/output, an auxiliary processor to perform floating point mathematical operations, a special-purpose microprocessor having an architecture suitable for fast execution of signal processing algorithms (e.g., digital signal processor), a slave processor subordinate to the main processing system (e.g., back-end processor), an additional microprocessor or controller for dual or multiple processor systems, or a coprocessor. Such auxiliary processors may be discrete processors or may be integrated with the processor **560**.

The processor **560** is preferably connected to a communication bus **555**. The communication bus **555** may include

a data channel for facilitating information transfer between storage and other peripheral components of the system **550**. The communication bus **555** further may provide a set of signals used for communication with the processor **560**, including a data bus, address bus, and control bus (not shown). The communication bus **555** may comprise any standard or non-standard bus architecture such as, for example, bus architectures compliant with industry standard architecture ("ISA"), extended industry standard architecture ("EISA"), Micro Channel Architecture ("MCA"), peripheral component interconnect ("PCI") local bus, or standards promulgated by the Institute of Electrical and Electronics Engineers ("IEEE") including IEEE 488 general-purpose interface bus ("GPIB"), IEEE 696/S-100, and the like.

System **550** preferably includes a main memory **565** and may also include a secondary memory **570**. The main memory **565** provides storage of instructions and data for programs executing on the processor **560**. The main memory **565** is typically semiconductor-based memory such as dynamic random access memory ("DRAM") and/or static random access memory ("SRAM"). Other semiconductor-based memory types include, for example, synchronous dynamic random access memory ("SDRAM"), Rambus dynamic random access memory ("RDRAM"), ferroelectric random access memory ("FRAM"), and the like, including read only memory ("ROM").

The secondary memory **570** may optionally include a internal memory **575** and/or a removable medium **580**, for example a floppy disk drive, a magnetic tape drive, a compact disc ("CD") drive, a digital versatile disc ("DVD") drive, etc. The removable medium **580** is read from and/or written to in a well-known manner. Removable storage medium **580** may be, for example, a floppy disk, magnetic tape, CD, DVD, SD card, etc.

The removable storage medium **580** is a non-transitory computer readable medium having stored thereon computer executable code (i.e., software) and/or data. The computer software or data stored on the removable storage medium **580** is read into the system **550** for execution by the processor **560**.

In alternative embodiments, secondary memory **570** may include other similar means for allowing computer programs or other data or instructions to be loaded into the system **550**. Such means may include, for example, an external storage medium **595** and an interface **570**. Examples of external storage medium **595** may include an external hard disk drive or an external optical drive, or and external magneto-optical drive.

Other examples of secondary memory **570** may include semiconductor-based memory such as programmable read-only memory ("PROM"), erasable programmable read-only memory ("EPROM"), electrically erasable read-only memory ("EEPROM"), or flash memory (block oriented memory similar to EEPROM). Also included are any other removable storage media **580** and communication interface **590**, which allow software and data to be transferred from an external medium **595** to the system **550**.

System **550** may also include a communication interface **590**. The communication interface **590** allows software and data to be transferred between system **550** and external devices (e.g. printers), networks, or information sources. For example, computer software or executable code may be transferred to system **550** from a network server via communication interface **590**. Examples of communication interface **590** include a modem, a network interface card ("NIC"), a wireless data card, a communications port, a PCMCIA slot and card, an infrared interface, and an IEEE 1394 fire-wire, just to name a few.

Communication interface **590** preferably implements industry promulgated protocol standards, such as Ethernet IEEE 802 standards, Fiber Channel, digital subscriber line ("DSL"), asynchronous digital subscriber line ("ADSL"), frame relay, asynchronous transfer mode ("ATM"), integrated digital services network ("ISDN"), personal communications services ("PCS"), transmission control protocol/Internet protocol ("TCP/IP"), serial line Internet protocol/point to point protocol ("SLIP/PPP"), and so on, but may also implement customized or non-standard interface protocols as well.

Software and data transferred via communication interface **590** are generally in the form of electrical communication signals **605**. These signals **605** are preferably provided to communication interface **590** via a communication channel **600**. In one embodiment, the communication channel **600** may be a wired or wireless network, or any variety of other communication links. Communication channel **600** carries signals **605** and can be implemented using a variety of wired or wireless communication means including wire or cable, fiber optics, conventional phone line, cellular phone link, wireless data communication link, radio frequency ("RF") link, or infrared link, just to name a few.

Computer executable code (i.e., computer programs or software) is stored in the main memory **565** and/or the secondary memory **570**. Computer programs can also be received via communication interface **590** and stored in the main memory **565** and/or the secondary memory **570**. Such computer programs, when executed, enable the system **550** to perform the various functions of the present invention as previously described.

In this description, the term "computer readable medium" is used to refer to any non-transitory computer readable storage media used to provide computer executable code (e.g., software and computer programs) to the system **550**. Examples of these media include main memory **565**, secondary memory **570** (including internal memory **575**, removable medium **580**, and external storage medium **595**), and any peripheral device communicatively coupled with communication interface **590** (including a network information server or other network device). These non-transitory computer readable mediums are means for providing executable code, programming instructions, and software to the system **550**.

In an embodiment that is implemented using software, the software may be stored on a computer readable medium and loaded into the system **550** by way of removable medium **580**, I/O interface **585**, or communication interface **590**. In such an embodiment, the software is loaded into the system **550** in the form of electrical communication signals **605**. The software, when executed by the processor **560**, preferably causes the processor **560** to perform the inventive features and functions previously described herein.

The system **550** also includes optional wireless communication components that facilitate wireless communication over a voice and over a data network. The wireless communication components comprise an antenna system **610**, a radio system **615** and a baseband system **620**. In the system **550**, radio frequency ("RF") signals are transmitted and received over the air by the antenna system **610** under the management of the radio system **615**.

In one embodiment, the antenna system **610** may comprise one or more antennae and one or more multiplexors (not shown) that perform a switching function to provide the antenna system **610** with transmit and receive signal paths.

In the receive path, received RF signals can be coupled from a multiplexor to a low noise amplifier (not shown) that amplifies the received RF signal and sends the amplified signal to the radio system **615**.

In alternative embodiments, the radio system **615** may comprise one or more radios that are configured to communicate over various frequencies. In one embodiment, the radio system **615** may combine a demodulator (not shown) and modulator (not shown) in one integrated circuit ("IC"). The demodulator and modulator can also be separate components. In the incoming path, the demodulator strips away the RF carrier signal leaving a baseband receive audio signal, which is sent from the radio system **615** to the baseband system **620**.

If the received signal contains audio information, then baseband system **620** decodes the signal and converts it to an analog signal. Then the signal is amplified and sent to a speaker. The baseband system **620** also receives analog audio signals from a microphone. These analog audio signals are converted to digital signals and encoded by the baseband system **620**. The baseband system **620** also codes the digital signals for transmission and generates a baseband transmit audio signal that is routed to the modulator portion of the radio system **615**. The modulator mixes the baseband transmit audio signal with an RF carrier signal generating an RF transmit signal that is routed to the antenna system and may pass through a power amplifier (not shown). The power amplifier amplifies the RF transmit signal and routes it to the antenna system **610** where the signal is switched to the antenna port for transmission.

The baseband system **620** is also communicatively coupled with the processor **560**. The central processing unit **560** has access to data storage areas **565** and **570**. The central processing unit **560** is preferably configured to execute instructions (i.e., computer programs or software) that can be stored in the memory **565** or the secondary memory **570**. Computer programs can also be received from the baseband processor **610** and stored in the data storage area **565** or in secondary memory **570**, or executed upon receipt. Such computer programs, when executed, enable the system **550** to perform the various functions of the present invention as previously described. For example, data storage areas **565** may include various software modules (not shown) that were previously described.

Various embodiments may also be implemented primarily in hardware using, for example, components such as application specific integrated circuits ("ASICs"), or field programmable gate arrays ("FPGAs"). Implementation of a hardware state machine capable of performing the functions described herein will also be apparent to those skilled in the relevant art. Various embodiments may also be implemented using a combination of both hardware and software.

Furthermore, those of skill in the art will appreciate that the various illustrative logical blocks, modules, circuits, and method steps described in connection with the above described FIG.s and the embodiments disclosed herein can often be implemented as electronic hardware, computer software, or combinations of both. To clearly illustrate this interchangeability of hardware and software, various illustrative components, blocks, modules, circuits, and steps have been described above generally in terms of their functionality. Whether such functionality is implemented as hardware or software depends upon the particular application and design constraints imposed on the overall system. Skilled persons can implement the described functionality in varying ways for each particular application, but such implementation decisions should not be interpreted as causing a departure from the scope of the invention. In addition, the grouping of functions within a module, block, circuit or step is for ease of description. Specific functions or steps can be moved from one module, block or circuit to another without departing from the invention.

Moreover, the various illustrative logical blocks, modules, and methods described in connection with the embodiments disclosed herein can be implemented or performed with a general purpose processor, a digital signal processor ("DSP"), an ASIC, FPGA or other programmable logic device, discrete gate or transistor logic, discrete hardware components, or any combination thereof designed to perform the functions described herein. A general-purpose processor can be a microprocessor, but in the alternative, the processor can be any processor, controller, microcontroller, or state machine. A processor can also be implemented as a combination of computing devices, for example, a combination of a DSP and a microprocessor, a plurality of microprocessors, one or more microprocessors in conjunction with a DSP core, or any other such configuration.

Additionally, the steps of a method or algorithm described in connection with the embodiments disclosed herein can be embodied directly in hardware, in a software module executed by a processor, or in a combination of the two. A software module can reside in RAM memory, flash memory, ROM memory, EPROM memory, EEPROM memory, registers, hard disk, a removable disk, a CD-ROM, or any other form of storage medium including a network storage medium. An exemplary storage medium can be coupled to the processor such the processor can read information from, and write information to, the storage medium. In the alternative, the storage medium can be integral to the processor. The processor and the storage medium can also reside in an ASIC.

The above description of the disclosed embodiments is provided to enable any person skilled in the art to make or use the invention. Various modifications to these embodiments will be readily apparent to those skilled in the art, and the generic principles described herein can be applied to other embodiments without departing from the spirit or scope of the invention. Thus, it is to be understood that the description and drawings presented herein represent a presently preferred embodiment of the invention and are therefore representative of the subject matter which is broadly contemplated by the present invention. It is further understood that the scope of the present invention fully encompasses other embodiments that may become obvious to those skilled in the art and that the scope of the present invention is accordingly not limited.

What is claimed is:

1. A system comprising:

an objective lens having an optical field of view;

a stage configured to support a sample;

at least one focusing line sensor positioned within a first optical path of the optical field of view and configured to receive a first portion of the optical field of view from the objective lens;

at least one imaging line sensor positioned within a second optical path of the optical field of view and configured to receive a second portion of the optical field of view from the objective lens while the at least one focusing line sensor receives the first portion of the optical field of view from the objective lens; and

at least one processor that controls one or more of the stage and the objective lens,

wherein, within the optical field of view, the first portion is spaced apart from the second portion by a distance h

which satisfies $0<h\leq\sqrt{R^2-(L/2)^2}$ and $h=v*t*M$, wherein R is a radius of the optical field of view, wherein L is a length of the at least one focusing line sensor, wherein v is a scan speed of the sample, wherein t is a time sufficient for the at least one processor to calculate a focus height from image data of the first portion and position the sample relative to the objective lens according to the calculated focus height, and wherein M is a magnification of the first portion.

2. The system of claim **1**, wherein the second portion is a center portion of the optical field of view.

3. The system of claim **2**, wherein the first portion is an off-center portion of the optical field of view.

4. The system of claim **3**, wherein the off-center portion is at a location ahead of a location of the center portion with respect to a scan direction.

5. The system of claim **1**, wherein the stage is a motorized stage, and the at least one processor controls the motorized stage.

6. The system of claim **1**, wherein the at least one processor further:

receives captured first image data for the first portion of the optical field of view from the at least one focusing line sensor;

determines a focus height from the captured first image data; and

causes the objective lens to move to the focus height prior to the first portion of the optical field of view being captured by the at least one imaging line sensor.

7. The system of claim **6**, wherein the at least one processor determines the focus height from the captured first image data by:

determining one or more pixels of best focus within the captured first image data received from the at least one focusing line sensor; and

determining the focus height that corresponds to the one or more pixels of best focus within the captured first image data received from the at least one focusing line sensor.

8. The system of claim **7**, wherein the one or more pixels of best focus are determined based on contrast.

9. The system of claim **1**, wherein the at least one focusing line sensor is tilted at an angle with respect to the rotation axis.

10. The system of claim **9**, wherein the center of the at least one focusing line sensor is aligned to the center of the at least one imaging line sensor along a Y-axis.

11. The system of claim **9**, wherein the at least one focusing line sensor comprises a line sensor array and a microlens array.

12. The system of claim **9**, wherein the at least one focusing line sensor comprises two or more focusing line sensors, wherein a first one of the two or more focusing line sensors is tilted in an opposite direction than a second one of the two or more focusing line sensors, and wherein the focus height is determined based on an average focus height derived from the first portion of the optical field of view captured by the first focusing line sensor and the first portion of the optical field of view captured by the second focusing line sensor.

13. The system of claim **1**, wherein the at least one focusing line sensor comprises a linear detector array and a wedge prism within an optical path between the objective lens and the linear detector array.

14. The system of claim **1**, wherein the at least one processor further aligns adjacent portions of image data into a single digital image of at least a portion of the sample.

15. The system of claim **1**, wherein the length L of the at least one focusing line sensor is longer than a length of the at least one imaging line sensor.

16. The system of claim **1**, further comprising a beam splitter configured to split the optical field of view into the first optical path and the second optical path.

17. The system of claim **1**, wherein the at least one focusing line sensor comprises a number of pixel sensors that is greater or less than a number of pixel sensors of the at least one imaging line sensor.

18. The system of claim **1**, wherein the sample comprises a microscope slide.

19. The system of claim **1**, wherein the at least one focusing line sensor comprises N camera lines, wherein $N\leq t*\kappa$, and wherein κ is a line rate of the at least one focusing line sensor.

20. A method comprising:

by an objective lens having an optical field of view, acquiring image data of at least a portion of a sample supported on a stage; and

by at least one beam splitter optically coupled to the objective lens, providing a first portion of the optical field of view, corresponding to a first portion of the sample, from the objective lens to at least one focusing line sensor, at an angle with respect to a rotation axis that is perpendicular to the optical axis, while providing a second portion of the optical field of view, corresponding to a second portion of the sample, from the objective lens to at least one imaging line sensor,

wherein, within the optical field of view, the first portion of the optical field of view is spaced apart from the second portion of the optical field of view by a distance h which satisfies $0<h\leq\sqrt{R^2-(L/2)^2}$ and $h=v*t*M$, wherein R is a radius of the optical field of view, wherein L is a length of the at least one focusing line sensor, wherein v is a scan speed of the sample, wherein t is a time sufficient to calculate a focus height from image data of the first portion of the optical field of view and position the sample relative to the objective lens according to the calculated focus height, and wherein M is a magnification of the first portion of the optical field of view.

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